NOTES ON THE PRESUMPTIVE TEST FOR B. COLI[1]

By Max Levine

Examinations of waters for B. coli constitute an important part of the tests employed for the control of water supplies. Isolation and complete identification of the organisms is too laborious and costly for routine work and it is therefore desirable to have some simple test by which the probable presence of B. coli may be quickly determined with a sufficiently high degree of reliability to give a reasonably accurate idea of the existence of pollution.

The ideal medium for such a presumptive test would be one in which B. coli flourishes while other forms are inhibited, but this ideal has not as yet been attained. The requirements of a reliable presumptive test may be briefly stated as follows:

1. The medium employed must be one in which a test characteristic of B. coli is quickly obtained and easily recognized.
2. The medium should not inhibit the growth of B. coli nor permit its overgrowth by such forms as B. aerogenes.
3. Anaerobic spore-forming gas producers should be inhibited or some simple supplementary test provided by which errors due to their presence may be eliminated.
4. It is desirable that the test should also differentiate between true B. coli and B. aerogenes.

Until 1906, the most commonly employed presumptive test was gas production in dextrose broth. The formation of 25 to 70 per cent gas, of which approximately one-third was CO_2 was regarded as an excellent indication of the presence of B. coli and dangerous pollution. This criterion has been conclusively discredited. The variability of the gas ratio as determined in routine analysis, the many bacterial forms which ferment dextrose but not laxtose, coupled with the relatively greater incidence of such forms in treated and partially purified sources than in polluted waters, makes the old dextrose broth presumptive test an unreliable index of pollution. This is particularly true in warm weather.

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Since 1906 the most commonly employed presumptive test has been lactose peptone bile. The advantages over dextrose broth are many, but recently there has been a tendency to use lactose broth in order to eliminate the inhibitory action of bile.

Where pollution has been recent, the lactose bile or lactose broth presumptive tests are very reliable, but with relatively pure or treated waters, a positive presumptive test is not infrequently obtained when no B. coli are present. This confusion is due to the presence of spore-bearing anaerobic lactose fermenters. The error may be easily eliminated by plating from the positive lactose bile or broth tubes to some solid medium in petri dishes, as recommended by the U. S. Treasury Department Standard for drinking waters on common carriers.

The recent work of Rogers and his associates of the United States Department of Agriculture, which has been confirmed by many other investigators, has demonstrated conclusively that there is a marked correlation between certain types of coli-like bacteria and their sources. Two types may be easily distinguished; the B. coli which is constantly found in feces of man and in sewage but rarely in unpolluted soil, and the B. aerogenes which is rarely obtained from feces, but commonly found in cropped soil, on grains, etc. That these two types are very different in their sanitary significance is evident, since B. coli is characteristically of fecal origin whereas B. aerogenes is not. It is therefore desirable that they be differentiated in routine water analysis.

The following procedure is suggested as routine:

1. Plant portions of the sample in 0.5 per cent lactose peptone broth. Incubate at 37°C. for forty-eight hours.

2. After twenty-four hours incubation smear onto eosine methylene blue agar plates described below, from the highest dilution showing any gas (preferably also from the next highest dilution) and incubate at 37°C. for twenty-four hours.

If gas production is due to B. coli, characteristic black colonies with a metallic lustre will develop on the eosine-methylene blue agar in fifteen to twenty-four hours. B. aerogenes also grows well on this medium but its colonies are so distinctly different from B. coli as to be easily distinguished. Anaerobic spore-forming gas producers will, of course, not develop, thus eliminating the error introduced by their presence in the fermentation tubes.

Lactose broth. The new Standard Methods of Water Analysis of the American Public Health Association recommend 0.5 per cent peptone and 1 per cent lactose for the lactose broth medium, and incubation for forty-eight hours before any confirmatory tests are applied. With 1 per cent lactose, this period of incubation is too prolonged and detrimental to the successful isolation of B. coli. In a private communication Dr. Joseph Race of Ottawa, Canada, points out that beginning with equal quantities of B. coli and B. aerogenes there are found many times as many B. aerogenes as B. coli in 1 per cent lactose peptone bile-salt broth after forty-eight hours. The ratio may be as high as 18 to 1. The probability of obtaining B. coli from such a mixture by plating on litmus lactose agar is evidently slight.

In some unpublished studies in this laboratory it has been observed that with pure cultures of B. coli a maximum count is obtained in about twelve hours. In a medium with 1 per cent lactose B. coli begins to die off after twenty-four hours, some strains disappearing very rapidly whereas many B. aerogenes-like forms do not. If the quantity of lactose is reduced to 0.5 per cent, the death of B. coli is retarded considerably, and the probability of its detection thereby increased. One-half of one per cent lactose is sufficient for rapid and characteristic fermentation and this quantity therefore seems more desirable than the standard 1 per cent.

Eosine methylene blue agar. The agar medium recommended is a modification of that employed by Holt-Harris and Teague for the isolation of B. typhi, and is prepared as follows: Distilled water, 1000 cc.: agar, 15 grams: peptone (Difco), 10 grams: K_2HPO_4, 2 grams.

Boil until dissolved. Make up loss due to evaporation, and place measured quantities in flasks or bottles. Sterilize in autoclave for fifteen minutes at 15 pounds pressure.

Neither adjustment of the reaction nor filtration is necessary.

For use the following materials are added to each 100 cc. of the melted agar as prepared above: 1 gram or 5 cc. of the sterile 20 per cent lactose solution, 2 cc. of 2 per cent aqueous yellowish eosine, and 2 cc. of 0.5 per cent aqueous methylene blue. The aqueous solutions of the dies will keep in the ice box several months.

Differentiation of B. coli and B. aerogenes. On the eosine methylene blue agar as prepared above, B. coli forms characteristic button-like colonies 2 to 4 mm. in diameter with large black centers. There is

also a greenish metallic lustre and they are only slightly raised above the surface of the medium. B. aerogenes forms colonies which are much larger, considerably raised above the surface of the medium: characteristically show a relatively small brown center and the metallic lustre is rarely observed.

Of 122 colonies tentatively diagnosed as B. coli from their appearance on the agar, 97 per cent were B. coli, while of 102 colonies of supposed B. aerogenes 83 per cent were confirmed. The differentiation on this medium therefore seems reasonably reliable.

Conclusions. In the lactose broth presumptive test, 1 per cent lactose is detrimental to the successful isolation of B. coli, as many strains die off rapidly after twenty-four hours. A reduction of the lactose to 0.5 per cent reduces this error and is therefore recommended for routine tests.

For a rapid confirmatory test, the modified eosine methylene blue agar, because of its simplicity, ease of preparation, and the differentiation which it permits between B. coli and B. aerogenes, may advantageously be substituted for litmus lactose agar or the Endo medium, in routine water analysis.

The necessity for confirming the presumptive test and differentiating between the objectionable B. coli and the more widely distributed B. aerogenes and anaerobic gas-formers is not only of theoretical interest but of considerable practical significance when dealing with surface waters which are purified by sedimentation or chlorination. Where the positive presumptive test is due to spore formers, the amount of chlorine necessary to remove them is far in excess of that required to make the water safe. B. aerogenes also seems to be more resistant to treatment than B. coli. A knowledge of the type of bacteria responsible for a positive presumptive test thus becomes of practical significance.